Intestinal integrity in broilers fed with ramie silage (boehmeria nivea) mixed with cassava bran (manihot esculenta)

Integridad intestinal en pollos de engorde alimentados con ensilaje de ramio (boehmeria nivea) mezclado con afrecho de yuca (manihot esculenta)

Jaime Andrés Betancourt Vásquez[1]
María Camila Guerrero Núñez[2]
Luz Andrea Estrada Núñez[3]

Abstract

Bird's intestinal health is determinant because of its connotation on disease control, but also because it is the source of utilization of nutrients that results in successful production. Conventional raw material used for the fabrication of concentrate is expensive and has low availability, for these reasons, food

1.Researcher, Faculty of Livestock Sciences, Research Group in Veterinary and Zootechnical Sciences, Unisarc, jaimeb@unisarc.edu.co.
2. Zootechnician.
3. M.V.Z,Teacher University of Caldas.

alternatives with indigenous resources such as forage, have been sought out. In this way, *B. nivea* stands out as an alternative for feeding birds. The goal of this study was to determine the effect of using silage from *B. nivea* mixed with cassava bran (Manihot esculenta) on the intestinal integrity of fed broilers. 60 male broilers of the Ross 308 line were located in 12 pens, to each of which a completely randomized experimental treatment design was given. The treatments consisted in two types of diets provided to the birds, a control group that was only given concentrate and another group, that additional to the concentrate used in the control group, was given *B. nivea* silage mixed with cassava bran. The results of this study indicate that the silage from *B. nívea* leaves was able to be used as an alternative to increase the height, width and depth of villi resulting in a greater digestion and absorption surface for nutrients.

Key words: tropical forage; Ramie; Intestinal health; Broilers

Resumen

La salud intestinal en las aves es determinante debido a su connotación en el control de enfermedades, pero también porque es la fuente de aprovechamiento de nutrientes que deriva en una producción exitosa. Las materias primas convencionales utilizadas en la fabricación de concentrados son costosas y de baja disponibilidad, por ello se han buscado alternativas de alimentación con recursos autóctonos como forrajes. De esta manera, el *B. nivea* se destaca como alternativa en la alimentación para aves. El objetivo de este trabajo fue determinar el efecto de la utilización de ensilaje de *B. nivea* mezclado con afrecho de yuca (*Manihot esculenta*) en la alimentación de pollo de engorde sobre la integridad intestinal. Se utilizaron 60 machos de engorde de la línea Ross 308 que fueron alojados en 12 corrales, a cada uno de los cuales fue asignado en un tratamiento según un diseño experimental Completamente al Azar. Los tratamientos consistieron en dos dietas suministradas a las aves, un control en

donde recibieron sólo concentrado y otra donde se suministró el concentrado del tratamiento control y adicionalmente se ofreció ensilaje de *B. nivea* mezclado con afrecho de yuca. Los resultados de este trabajo indican que el ensilaje de las hojas de *B. nívea* se puede utilizar como alternativa para aumentar la altura, ancho y profundidad de las vellosidades dando como resultado una mejor superficie para la digestión y absorción de los nutrientes.

1. Introduction

The importance of a good intestinal health in birds is determinant, not only because of its clinical connotation on disease control but also because digestion processes are complemented in the small intestine, in which epithelial cell linings absorb products from digestion (Pacheco et al., 2008). Intestinal villi are in charge of providing a large internal surface, increasing the area of intestinal absorption and digestion. The size, length and width can vary throughout the small intestine because of the specific function of each portion (Boleli et al., 2002). For this reason, intestinal integrity is the source of utilization of nutrients that results in successful production. In this sense, animal nutrition has been a productive pillar with a wide investigation field, seeking to improve digestibility of raw materials available through ingredient processing or complete balanced meals (Mahagna *et al*, 1995; Sell, 1996).

Taking into account that conventional raw materials used in the fabrication of balanced meals are expensive and have little availability, food alternatives with indigenous resources such as forage, have been sought out., which represent an important source of nutrients for animals. Different studies have demonstrated that supplementation with lead flour in aviculture, decreases production costs and improves profit margin (Betancourt et al., 2017). For these reasons, preservation and conservation of these raw materials with different practices such as silage, haymaking, and pelleting, is recommended (Ledesma, *et al,* 2002).

The Ramie (Boehmeria nivea) has different uses, of which animal feeding stands out because it accumulates as much N in its leaves as is found in legumes, it has rapid growth and results appetizing for poultry, cattle and pork (Moranet et al., 2012). Although *B. nívea* has low non-fibrous carbohydrate content, the cassava bran mix *(Manihot esculenta),* that has a great NFC but little protein results of great utility to compensate the deficit of NFC of the forage and of the bran protein, generating an adequate nutritional balance of this meal (Betancourt et al., 2017).

For the reasons stated above and taking into account physiologic studies that have demonstrated that, birds adapt their intestinal tract's function to maximize food digestion and nutrient absorption according to the characteristics of the digestive content (Mateos et al., 2002), it is necessary to have knowledge on the effect that, meals with forage rich on fiber, have on the internal intestinal conformation and the absorption of nutrients. The goal of this study was to determine the effect of using silage from *B. nivea* mixed with cassava bran (*Manihot esculenta*) on the intestinal integrity of fed broilers evaluating the height, width and depth of intestinal villi.

2. Materials and Methods

Location.

The investigation took place in the Cattle Farm of the University Corporation Santa Rosa de Cabal, UNISARC. El Jazmín University Campus, located at 4°52'07" LN and 75°37'22" LO, Kilometer 4 via Santa Rosa de Cabal- Chinchiná (Caldas), located at 1640 meters above sea level, with an average temperature of 18.6°C, annual rainfall of 2620 mm and a relative humidity of 72%.

The experiment was carried out in the period between April and July 2017.

Experimental period and treatments.

The experiment lasted 42 days (all the birds were received on their first day of life and had the same management, weight evaluation and uniformity until sacrifice). The treatments consisted of two diets (T2) commercial concentrate and treatment (T1) consisting of Ramie silage *(Boehmeria nivea)* mixed with cassava bran (Manihot esculenta). It was given at a 70:30 ratio and administered at will between 5:00 p.m. and 7:00 a.m. of the following day, during this time the concentrate was removed to stimulate the consumption of forage. Table (1).

Table 1. *Chemical composition of the silage and concentrate used in the experiment***.**

Chemical composition	Concentrate	Ramie silage + cassava
Dry Matter (DM) g/kg	880.9	317.8
Raw Protein (RP) g/kg	216.2	141.7
NDF, g/kg MS	96.7	201.9
FDA, g/kg MS	51.2	137.3
NSC[1] , g/kg MS	-	524.5
Lignin g/kg	17	81.5
Ethereal extract, % MS	-	8.1
Ashes, % MS	64.7	123.8

Animals and treatment.

60 male broilers of the Ross 308 line (1 day of age), were used, and were located in 12 pens. The experimental unit consists of cages of 5 animals, finishing the experiment with 4 birds/m2. 1 m^2 cement floor cages were used, they had a bed of chips and brick walls equipped with two Nipple-type trough and a hopper-type birdfeeder. Each pen was assigned to the treatments, according to a completely randomized experimental design of 2 treatments with 6 repetitions. Gas brooders (1 brooder / 6 pens) were used

to control the temperature of the shed during the first 21 days (adjusting the temperature to 30°C during the first 2 days until reaching 23°C on day 27). The animals were vaccinated against Newcastle La Sota (on day 6 and 14; ocular) and Gumboro (on day 17; orally). A 23 h light regime was handled during the first 7 d and 18 h until sacrifice. The sacrifice was made by cervical dislocation and all the processes and procedures of the experiment were approved by the Bioethics Committee of the University Corporation Santa Rosa de Cabal-Unisarc.

Elaboration of the diet with Ramie silage (Boehmeria nivea.

The silage was elaborated with Ramie leaves. To make the silo, the tree branches were cut off at 10:00 a.m., stripped by hand and left in the sun until 4:00 p.m. to reduce humidity. The leaves were chopped (approximately 2.5 cm) in a pickaxe (Trapp® TRF-300), 20% of the material to be ensiled was deposited (20 kg), it was stepped on to eliminate the air and the additive was added (1 L); This process was repeated 5 times until the silage was completed, at which time the forage was covered with plastic and enough sand was deposited on it to allow the lid to close and avoid air chambers in the silo. Cane molasses mixed with fermented juice of epiphytic flora (ratio 4:1; w/v) was used as an additive. The fermented juice was prepared by adapting the description by Bureenok *et al*. (2006) as follows: 200 g of fresh forage were macerated in 1 L of H2O d with a blender, the macerated material was filtered through two layers of gauze, the juice was mixed with cane sugar (30 g of L-1 sugar), it was deposited in an amber glass bottle avoiding air chambers, it was shaken and left to rest for 18 h at room temperature.

Collection of intestinal samples for laboratory

After evisceration, the components of the digestive system were separated to weigh the duodenum (from the pylorus to the distal portion of the duodenal turn), the jejunum (from the

duodenum to Meckel's diverticulum) and the ileum (from the jejunum to the beginning of the cecum); additionally, the length of the duodenum, the jejunum and the ileum were measured. For the histological analysis, a 3 cm segment of the terminal portion of the duodenum, the jejunum and the ileum were sectioned and washed with saline solution. Tissue samples were deposited in 10% buffered formalin for 18 h, a ratio of 1:10 (sample: formalin), washed 3 times in H2O dd, placed in 70%(v/v) ethanol and then the tissues were subjected to histological routine with the inclusion of paraffin material. With the use of a microtome, 8 cuts of 7 µm of thickness marked with hematoxylin and eosin were made; In these cuts and with the help of an optical microscope coupled to an image analysis system, the height (distance between the beginning of the basal area coinciding with the upper portion of the crypt to the apex) and width of the villi were measured, as well as the depth of the crypts and the thickness of the muscular layer in the different segments of the intestine. Twenty measurements were made for each collective tissue.

Chemical and microbiological analysis

The concentration of moisture, ash and ethereal extract was determined in the concentrate and silage (with and without cassava bran by gravimetry (AOAC 200.18 and AOAC 9942.5 methods, respectively). Raw protein (RP) was determined by the Kjeldahl method (Thiex et al., 2002), neutral detergent fiber (NDF), and FDA acid detergent fiber (Van Soest et al., 1991) were also determined. Non-structural carbohydrates (NSC) were estimated according to NRC (1998). A microbiological examination was also carried out for the silage (See Annex E). In addition to this, ileum and cloaca swabs were performed in order to determine the health of the lot before the beginning of the treatments and they did not report pathogenic bacteria, also a water analysis presented compliance according to the established parameters.

Histological analysis of intestinal samples

The sample was fixed for 18 hours in 10% buffered formalin to protect it from the environment, a 3 cm sample was taken, then it was passed to a hydration machine, the histological tissue processes were carried out and later it was mounted in paraffin, to be taken to the Leica RM 2135 microtome cutter and later stained with hematoxylin-eosin; The plate is mounted, placed on a sheet and analyzed in the Laser LASEZ- Leica equipment with an ICCC50 HD camera for visualization and software for measuring height, width and depth, with a 40, 45 and 100x lens.

Statistical analysis

With the data obtained in the field, a database was built in the Excel program, which was exported to the statistical program S.A.S (Statistical Analisys System) v. 8.0 (Copyright © 1985-2003A test was carried out to check the assumption of normality of the data, through the statistic Shapiro Wilk at 5%, finding that in all the variables and tissues the assumption of normality was violated; due to the above, the data was analyzed by Non-Parametric Statistics using the Kruskal Wallis test.

3. Results and discussion

Table (2) shows the effect that T1 and T2 has in each section of the small intestine comparing the size of its villi and its degree of significance where:

Tabla 2. *Intestinal integrity in broilers fed with Ramie silage (Boehmeria nivea) mixed with cassava bran (Manihot esculenta)*

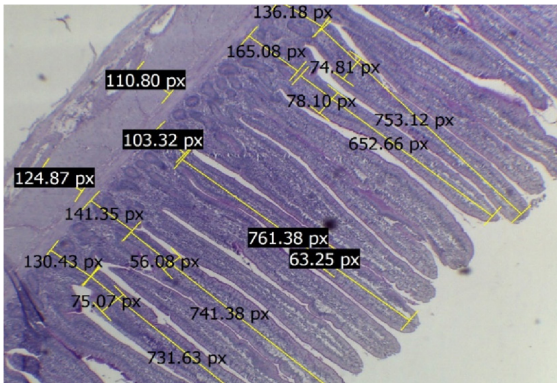
SECTION	TREATMENT	HEIGHT	WIDTH	CRYPT
DUODENUM	Silage	1004.92 ± 249.81	71.09 ±9.67	310.25 ± 83.07
	Control	918.39 ±126.65	66.55 ±19.92	205.03 ± 63.23
	Kruskal Wallis	0.9135	0.7218	6.4962
	Significe	0.3300 NS	0.3956 NS	0.0108 *
JEJUNUM	Silage	594.72 ± 75.58	65.19 ±21.39	157.62 ± 59.96
	Control	743.01 ±144.53	77.40 ± 22.63	166.70 ± 49.10
	Kruskal Wallis	9.2084	1.8191	1.3272
	Significance	0.0024 **	0.1774 NS	0.2493 NS
ILEUM	Silage	1020.16 ±1234.84	79.28 ± 24.61	158.95 ± 48.80
	Control	581.24 ± 77.79	66.77 ±27.08	127.25 ± 21.52
	Kruskal Wallis	7.7368	3.5013	4.8496
	Significance	0.0054 **	0.0613 NS	0.0277 *

NS = No significant difference (P>0.05), (*) = There is a significant difference (P<0.05),

(**) = There is a highly significant difference (P<0.01).

In the duodenum, between the treatments, no significant differences were found in the height and width of the villi (P>0.05); in the crypt there was a significant difference of 0.0108 presenting itself better in the T1 of the experiment (Figure 1).

Figure 1. Duodenum of broilers fed *with Ramie silage (Boehmeria nivea) mixed with cassava bran (Manihot esculenta)*



The orange line indicates the height of the villi, the red line indicates de width of the villi and the green line indicates the depth of the crypt. 4x magnification.

According to Giannenas *et al*., 2012; Salim *et al*., 2013 y Yu *et al*., 2011, the height of intestinal villi increased and the depth of the crypts rapidly decreased after the hatching, this increased the absorption surface of nutrients. The birds fed with a baseline diet presented deeper and wider crypts, this implies a greater requirement because of the high cell turnover to maintain this tissue.

It has been suggested that villi of greater height make for a better digestion and absorption surface for nutrients (Gómez *et al*, 2018). The data obtained from this study shows higher villi in the jejunum section in T1, resulting in a better surface. Intestinal villi are the structures found at the level of the intestinal mucosa that serve as a reference to determine the efficiency in the absorption of nutrients.

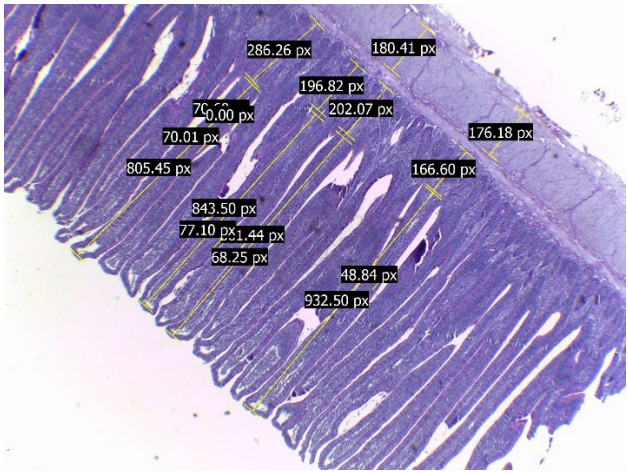
Compact and well heighted villi are indicative of better available nutrient absorption (Caspary, 1992 cited by Cao, 2013) and is associated with active cellular mitosis (Samanya y Yamauchi, 2002 cited by Disaji et al., 2013). The comparisons made between stablished treatments, showed that there was a significantly higher difference in the height of the jejunum villi compared to the control group in 0.0024.

This concept is supported with the result obtained by comparing the measurement of villi height of the control group with the group who received silage and concentrate at the same time, where significant differences were found with averages of between 1 and 5. The increase in the depth of the crypts translated in the increase of cell rotation, resulting in a rapid renovation of the villi that could be necessary during the increase of pathogenic load (Awad *et al*., 2009). When analyzing between treatments,

an increase in the depth of the duodenum and ileum crypts were observed, showing significant differences compared to the jejunum.

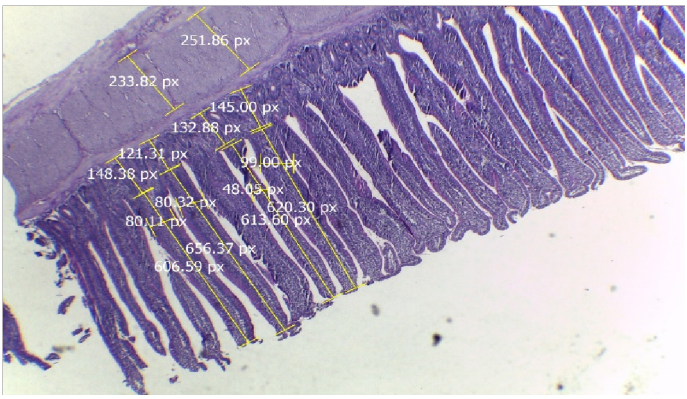
The jejunum shows no significant differences, between treatments, for the width and depth of the crypt (P>0.05); as for the height, it presents highly significant differences in 0.0024 corresponding to (P<0.001), in T1 compared to the control (Figure 2).

Figura 2. *Jejunum of broilers fed with Ramie silage (Boehmeria nivea) mixed with cassava bran (Manihot esculenta)*



The ileum does not present significant differences in the width of the villi between treatments (P>0.05); as for height, it presents a highly significant difference for T1 compared to T2 (P<0.001) and the crypt present significant differences for T1 (P<0.05) (Figure 3).

Figure 3. *Ileum of broilers fed with Ramie silage (Boehmeria nivea) mixed with cassava bran (Manihot esculenta)*



The analysis of variance shows that there were highly significant differences in villi height and crypt depth in the duodenum, jejunum and ileum, among the treatments. In the Kruskal Wallis test it was found that silage consumption showed statistical differences in the duodenum and ileum; The control treatment presented greater height of jejunum villi.

There is a close correlation between crypt depth and epithelial cell proliferation rates. In addition, the number of proliferations and epithelial cell turnover have a great impact on protein and energy requirements of the small intestine mucosa (Yasar *et* al, 1999;Hampson *et al* ,1986). The above correlates with the results obtained in this study because T1 has a significant difference between ($P<0.05$) in the depth of the duodenum and ileum crypts and a significantly high difference ($P<0.001$) in the height of jejunum and ileum villi compared to T2. Between the villi, structures called crypts of Lieberkühn, are observed where there are also Paneth cells, characterized for their pyramidal shape and whose function are protein synthesis. (Khalid *et al*, 2012). Birds have an efficient digestive capacity because they use 60 to 70% of the nutrients contained in the diet (Antillón et al., 1987). The percentage is consistent with each ingredient of the meal (Mack, 1986).

4. Conclusions

The results of this study indicate that silage from *B. nívea* leaves can be used as an alternative to increase the height and width of villi in the jejunum and duodenum section respectively, resulting in a better surface for digestion and absorption of nutrients.

The consumption of silage from *B. nívea* leaves, increased the size of villi and the height and depth of the crypt in the duodenum, jejunum and ileum portions, favoring the efficient digestive capacity of the birds that already use around 70% of the nutrients contained in the diet.

References

Antillon, R,A y López, C.C (1987). *Enfermedades Nutricionales de las Aves*. (1era. Edicion). Universidad Nacional Autonoma de Mexico. (pp. 338,357-360). Mexico: D.FoAwad W.A.,

Bo¨hm J, Razzazi-Fazeli E., Ghareeb K, Zentek (2006). Effect of Addition of a Probiotic Microorganism to Broiler Diets Contaminated with Deoxynivalenol on Performance and Histological Alterations of Intestinal Villi of Broiler Chickens.

AOAC (2010). Official methods of analysis of AOAC international, 18ra Ed. Association oficial to Analytical Chemistry, United States.

Dizaji, B et al. (2013) Influences of different growth promoters on intestinal morphology of broiler chickens. Tabriz, Iran

Garcés Molina, Adelaida María; Berrio Roa, Lorena; Ruiz Alzate, Santiago, Serna de León, Juan Guillermo; Builes Arango, Andrés Felipe, 2004 Ensilaje como fuente de alimentación para el ganado. *Revista lasallista de investigación*, *1*(1), 66-71.

Giannenas, I.; Papadopoulos, E.; Tsalie, E.; Triantafillou, E.; Henikl, S.; Teichmann, K. and Tontis, D. 2012. Assessment of dietary supplementation with probiotics on performance, intestinal morphology and microflora of chickens infected with Eimeria tenella. *Vet Parasitol, 188*, 31-40.

Hampson DJ, Kidder DE. Influence of creep feeding and weaning on brush border enzyme activities in the piglet small intestine. *Res vet sci 40*(1):24-31.

Junqueira L.C, Carneiro J. (2005). *Basic Histology: Text & Atlas.* McGraw-Hill's Access Medicine,

Ledesma, L., Gallego, L., & Peláez, F. J (2002). Situación actual de la ganadería de carne en Colombia y alternativas para impulsar su competitividad y sostenibilidad. *Revista Colombiana de Ciencias Pecuarias, 15*(2), 213-225.

Mack, O. N. (1986). *Digestión y metabolismo cap. 24. Manual de producción avícola* (Traducción de la tercera edición). (pp. 525-529). México: D.F: El Manuel moderno

Mahagna, M Nir, I; Larbier, M., & Nitsan, Z (1995). Effect of age and exogenous amylase and protease on development of the digestive tract, pancreatic enzyme activities in Young meat- type chicks. *Reproduction Nutrition Development, 35* (2), 201-212.

Thiex, N.; Manson, H.; Anderson, S. y Persson, J.-A. (2002). Determination of crude protein in animal feed, forage, gain, and oilseeds by using block digestion with a copper catalyst and steam distillation into boric acid: collaborative study. J. *AOAC.int.85*(2):309-317.

Uni Z., Ganot S. y Sklan D. (1998). Posthatch development of mucosal function in the broiler small intestine. *Poultry. Sc; 77*: p 75-82.

Van Soest, P.J.; Robertson, J.B y Lewis, B.A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74(10):3583-3597.

Yasar s, Forbes JM. (1999).Performance and gastrointestinal response of broiler chickens fed on cereal grain-based feeds soaked in water. *Br Poult sci 40*:65-7.